

Phylogeny and Speciation in Pinnipedia and Cetacea — A Cytogenetic Study

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The karyology of the 2 mammalian orders Pinnipedia and Cetacea was recently reviewed by ÁRNASON (1974a, b) who presented both his own results, a survey of relevant papers and pertinent karyological work in progress. Chromosome studies carried out earlier by using conventional staining methods showed that both orders were characterized by a karyotypic uniformity, which is not paralleled elsewhere in mammals (ÁRNASON 1972). In order to obtain a more definite basis for evaluation of the degree of agreement, the karyotypes were compared, in the 2 papers referred to above (ÁRNASON 1974a, b), by means of banding methods and autoradiography. In the present survey of the karyology of Cetacea and Pinnipedia, each order will first be reviewed separately; thereafter, some relevant characteristics of both orders will be discussed.

Karyology of the Pinnipedia

The order Pinnipedia comprises 32 species of recent seals, sea lions and walruses (KING 1964). The superfamily Otarioidea which is divided into the Otariidae and the monotypic family Odobenidae comprises 14 species. The superfamily Phocoidea consists of a single family, the Phocidae, comprising 18 species, including the possibly extinct West Indian monk seal, *Monachus tropicalis*. At least 5 different species of otariids, the walrus, and 16 phocid species have now been studied karyotypically by various workers. The species studied and their chromosome numbers are given in Table 1 together with the pertinent references.

The range of variation of chromosome numbers in the Pinnipedia is low, from $2n=32$ in some of the phocids and in the walrus to $2n=36$ in the otariids. Excepting the walrus karyotype, all karyotypes within each chromosome number group are in close agreement. The karyotype of the walrus has a separate position

and cannot be compared directly to the other karyotypic groups.

FAY et al. (1967) studied the karyotypes of 6 species of pinnipeds (*Callorhinus ursinus*, *Eumetopias jubatus*, *Odobenus rosmarus*, *Leptonychotes weddelli*, *Erignathus barbatus* and *Phoca vitulina*). After comparisons of the karyotypes and counts of the fundamental numbers of these species and of 2 additional species from the literature, viz. *Pusa hispida* (COREMAN and RICHART 1964) and *Zalophus californianus* (HUNGERFORD and SNYDER 1964), they concluded that the results were compatible with the theory of a monophyletic origin of the Pinnipedia.

The direct relationship between the 34 and 32 chromosome phocid karyotypes was pointed out by ÁRNASON (1970a), who suggested that two pairs in the 34 chromosome karyotype, viz. one small satellited pair, designated sat, and the only pair in the telocentric group, t, (nomenclature according to LEVAN et al. 1964), had given rise through fusion to a large satellited pair, m1, in the 32 chromosome karyotype. It seemed reasonable to consider the sat and t pairs as preceding the m1 pair, since in the Canioidea (e.g. WURSTER and BENIRSCHKE 1968), from which the Pinnipedia have evolved, similar chromosomes are found, whereas the m1 chromosome is unique for the $2n=32$ phocids.

The G-band studies performed on the 34 and 32 chromosome phocid karyotypes showed virtually homologous banding patterns both within and between these groups. The G-band patterns of the sat, t and m1 pairs provided unequivocal evidence for the relationships indicated above. Thus, homologous banding was found in the long arm of the m1 and t chromosomes, and in the short arm of m1 and in the sat chromosome; in the latter case the banding showed that the terminal region of the short arm of the sat chromosome had fused with the short arm of the t chromosome. After the relationship between the 34 and 32 chromo-

Table 1. Chromosome numbers of pinnipeds

Family	Species	♀	♂	2n	Ref. no
Otariidae	<i>Eumetopias jubatus</i>	+	+	36	4, 7, 8
	<i>Zalophus californianus</i>	+	+	36	4, 5, 8, 10
	<i>Arctocephalus australis</i>	+	+	36	12
	<i>Arctocephalus philippi</i>	+	+	36	8
	<i>Callorhinus ursinus</i>	+	+	36	7, 8, 11
Odobenidae	<i>Odobenus rosmarus</i>	+	+	32	4, 7, 8, 11
Phocidae	<i>Lobodon carcinophagus</i>	+	+	34	13
	<i>Ommatophoca rossi</i>	+	+	34	9
	<i>Hydrurga leptonyx</i>	+	+	34	9
	<i>Leptonychotes weddelli</i>	+	+	34	2, 4, 7, 9
	<i>Mirounga leonina</i>	+	+	34	9
	<i>Mirounga angustirostris</i>	+	+	34	4, 8, 11
	<i>Monachus schauinslandi</i>	+	+	34	8
	<i>Erignathus barbatus</i>	+	+	34	4, 7, 8
	<i>Cystophora cristata</i>	+	+	34	4, 8, 12
	<i>Phoca vitulina</i>	+	+	32	4, 7, 11, 12
	<i>Pusa hispida</i>	+	+	32	4, 6
	<i>Pusa caspica</i>	+	+	32	1
	<i>Pusa sibirica</i>	+	+	32	1
	<i>Halichoerus grypus</i>	+	+	32	3, 4
	<i>Pagophilus groenlandicus</i>	+	+	32	1, 4
	<i>Histriophoca fasciata</i>	+	+	32	4, 8

1. ANBINDER (1969, 1971)

2. ARAKAKI and KENNEY (1970)

3. ÁRNASON (1970a)

4. ÁRNASON (1974a)

5. BENIRSCHKE (pers. comm.)

6. COREMAN and RICHART (1964)

7. FAY et al. (1967)

8. FAY (in prep.)

9. HOFMAN (in prep.)

10. HUNGERFORD and SNYDER (1964)

11. KULU (1972)

12. PFITZER and BLESSING (1969)

13. SEAL et al. (1970)

some phocid karyotypes had been established, the otariid karyotypes were compared with the 34 chromosome phocid karyotypes by means of the G-band technique. The results showed that the majority of the pairs had identical or virtually identical G-band patterns. These facts unequivocally indicate a common evolutionary lineage and strongly support the theory of monophyletic origin of the Pinnipedia.

A comparison of the chromosomes of Caniidea and Pinnipedia revealed that the greatest similarities exist between the pinniped and procyonid karyotypes, both as regards chromosome number and characteristic chromosomes. The chromosome number of the procyonins is $2n=38$ and chromosomes with a morphology similar to that of the pinniped sat and t chromosomes are found in the procyonid karyotypes. Even though it cannot be definitely

determined whether or not the $2n=36$ otariid karyotype is older than the $2n=34$ phocid karyotype, it may be assumed tentatively that the $2n=36$ otariid karyotype is primordial to the $2n=34$ chromosome phocid karyotype because of its greater resemblance to the procyonid karyotypes.

Variance analysis of measurements of homologous chromosomes of four $2n=34$ and five $2n=32$ phocid species demonstrated a very high degree of agreement between the different species. However, one species, *Erignathus barbatus*, showed values that deviated clearly from those of the other species.

The C-band studies performed on the phocid karyotypes revealed that *Erignathus* differed from the other species studied both in amount and distribution of C-heterochromatin. In *Erignathus*, distinctly stained bands were seen in

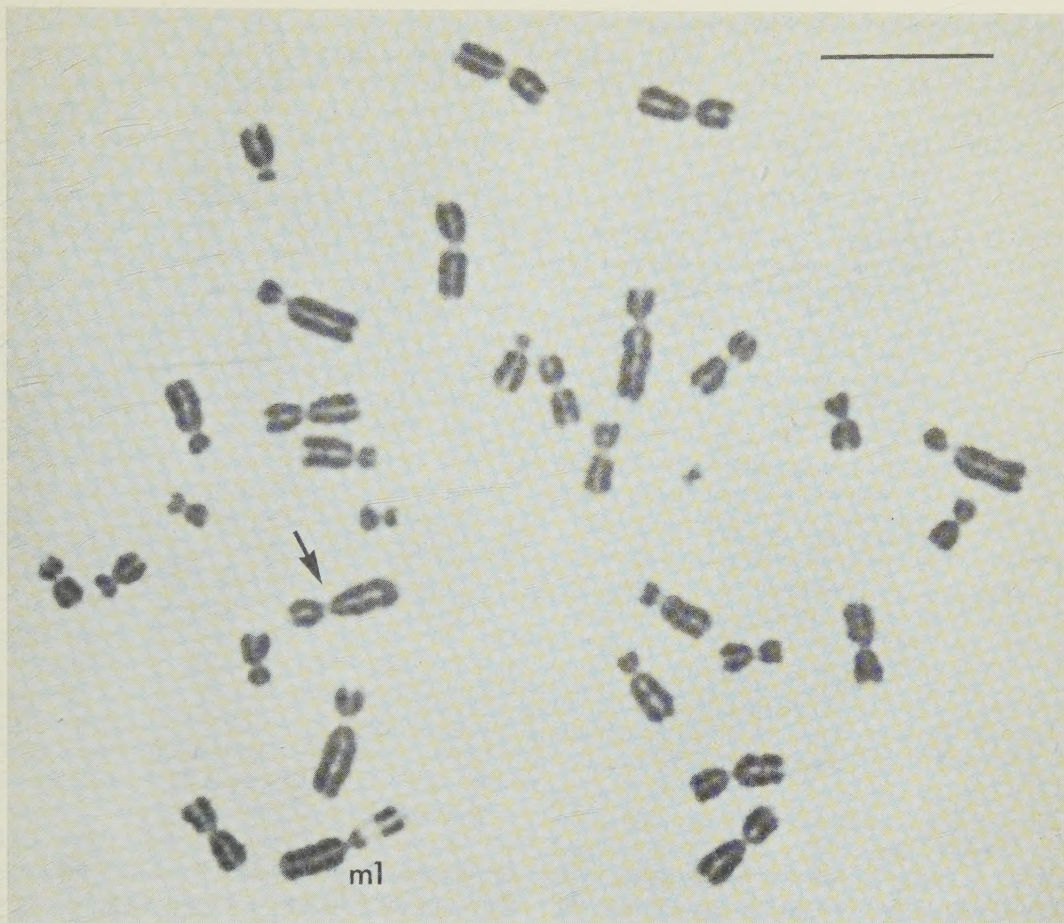


Fig. 1. Metaphase chromosomes of a male harbour seal, *Phoca vitulina*. The secondary constriction in the presumptive homologue (arrow) of m1 was not present in this specimen. Scale: 10 μ .

the centromeres of most pairs of the complement. C-bands of lower staining intensity were also present in terminal positions on the short arms of a few pairs. In the other species studied, C-bands were very sparse and were strictly limited to the centromeres, except for a C-band occasionally seen in the secondary constriction of m1 in the 32 chromosome species. The statistical calculations based on chromosome measurements thus coincided with the cytological findings in the Phocidae. The karyological results indicate that *Erignathus* is related more distantly — and *Cystophora* more closely — to the Phocini than usually claimed in classical systematics.

With one exception, the X of the pinnipeds measured 5—5.5 % of the female haploid set. The exception was *Histiophoca fasciata*, in which the X measured 8.8 %. In this species the distal part of the long arm of X is heterochromatic, which means that the relative length of the facultative heterochromatin of *Histiophoca* is approximately the same as in other phocids.

Of the 40 pinnipeds studied, one animal showed an intraspecific karyotype deviation. The animal in question was a male pup of *Phoca vitulina* taken in Fljótshólar, Iceland. Fig. 1 shows the chromosomes of this specimen. The secondary constriction in chromosome m1 was

Table 2. Chromosome numbers of cetaceans

Suborder	Species	♀	♂	2n	Ref. no
Odontoceti	<i>Inia geoffrensis</i>		+	44	11, 12
	<i>Stenella dubia</i>		+	44	4
	<i>Tursiops truncatus</i>	+	+	44	9, 14, 15
	<i>Tursiops gilli</i>	+		44	4
	<i>Delphinus delphis</i>	+	+	44	4, 11, 12
	<i>Lagenorhynchus obliquidens</i>	+	+	44	9
	<i>Globicephala macrorhyncha</i>	+	+	44	4, 15
	<i>Orcinus orca</i>	+	+	44	8, 10, 11, 12
	<i>Phocoena phocoena</i>	+	+	44	4, 7
	<i>Phocoenoides dalli</i>		+	44	11, 12, 13
	<i>Monodon monoceros</i>		+	44	1
	<i>Physeter catodon</i>		+	42	3, 5, 6
Mysticeti	<i>Kogia breviceps</i>		+	42	5
	<i>Eschrichtius gibbosus</i>	+		44	4, 11
	<i>Balaenoptera acutorostrata</i>	+	+	44	4
	<i>Balaenoptera borealis</i>	+	+	44	3, 4
	<i>Balaenoptera physalus</i>	+	+	44	2, 4, 11

1. ANDREWS et al. (1973)

2. ÁRNASON (1969)

3. ÁRNASON (1970b)

4. ÁRNASON (1974b)

5. ÁRNASON and BENIRSCHKE (1973)

6. ATWOOD and RAZAVI (1965)

7. BENIRSCHKE (pers. comm.)

8. CARR et al. (1969)

9. DUFFIELD et al. (1967)

10. HORRALL et al. (1968)

11. KULU (1972)

12. KULU et al. (1971)

13. MAKINO (1948)

14. PRASAD et al. (1970)

15. WALEN and MADIN (1965)

visible in only one of the homologues, indicating a complete deletion of this region in the other. This was supported by observation of telophase cells, which had only one nucleolus as compared with two in the control material. Apparently, the nucleolus is formed in the secondary constriction region of m1. No anatomical deviations were observed in the pup.

Karyology of the Cetacea

The number of cetacean species recognized by different authorities varies slightly. In a recent review of cetacean karyology, the present author (ÁRNASON 1974b) adopted the scientific nomenclature of RICE and SCHEFFER (1968), who distinguish 69 odontocete and 10 mysticete species. Their view, however, that Odontoceti (toothed whales) and Mysticeti (whalebone whales) are separate orders was

not upheld by the results of the karyological studies of the Cetacea.

So far, karyotypes of at least 17 cetaceans, 13 odontocetes and 4 mysticetes, have been described in the literature (Table 2). In the Odontoceti the material studied represents 4 of the 5 families, viz. Platanistidae, Delphinidae, Monodontidae and Physeteridae. Thus, the karyotypes of only one odontocete family, the Ziphiidae, are still unknown. In the Mysticeti, material from the families Eschrichtiidae and Balaenopteridae has been studied. The only family so far unknown karyotypically is the Balaenidae.

The first cetacean species of which the karyotype was studied was the Dall porpoise, *Phocoenoides dalli*, reported by MAKINO (1948). MAKINO based his analysis on direct preparations of male germ cells and determined the chromosome number as $2n=44$, including one heteromorphic pair that was selected as the sex chromosomes. WALEN and MADIN (1965)

studied the chromosomes of two odontocetes, the bottlenosed dolphin, *Tursiops truncatus* and the pilot whale, *Globicephala scammoni*; they used cells grown in cell culture for their analyses. The first mysticete karyotype studied with the use of cell culture was that of the fin whale, *Balaenoptera physalus*, (ÁRNASON 1969). The chromosome number of this species, too, was $2n=44$. In his paper, ÁRNASON advocated the theory of a monophyletic evolution of the Odontoceti and Mysticeti on the basis of the similarities between the fin whale karyotype and those of the bottlenosed dolphin and pilot whale. This hypothesis has been continuously supported by the accumulating knowledge of cetacean karyology.

The general cetacean karyotype is characterized by 4 t pairs and 2—4 large st pairs. The largest sm pair in all karyotypes stands out because of its size. The m chromosomes are all rather small, having a maximum size of approximately 5 % of female haploid set. The homologues of the smallest sm pair are frequently attached to each other by their short arms, indicating that they participate in the formation of the nucleolus. Similar attachments between chromosomes of the t group are also common. The X chromosomes of the odontocete karyotypes measured 5—5.5 % of the female haploid set. The X chromosomes of the minke and sei whales measured 4.7 %. The X of the fin whale was unusually large, measuring approximately 8.5 %.

In preparations stained by conventional staining methods, size heteromorphism is occasionally observed in the largest pairs. It is not excluded that size heteromorphism occurs in other pairs too; the similar morphology of many of the smaller pairs would make it impossible to detect such heteromorphism, however.

Three species do not fit into the pattern of the general cetacean karyotype. These are the sperm and pygmy sperm whales, both having $2n=42$, and the killer whale, having $2n=44$. The karyotypes of the sperm and pygmy sperm whales are entirely different from the general cetacean karyotype, which obviates any comparison between the two types. The killer whale has only one t pair and thus lacks the

main characteristics of the general cetacean karyotype. Since, however, the other features are similar to those of the general cetacean karyotype, the difference may be explained either by accumulation of heterochromatin in the short arms of t chromosomes or by pericentric inversions.

The application of the Q- and G-banding methods to the cetacean karyotypes was of great help for establishing correct chromosome pairing and identifying the X. These methods, however, were not used as a basis for a detailed comparison between odontocete and mysticete karyotypes, since it is hardly feasible to compare banding patterns of different species with complete objectivity unless they display a high degree of banding homology.

The C-banding of the 7 cetaceans studied yielded a striking general picture in all species, and seems to be unique among mammals in certain respects. The amount of C-heterochromatin in the 4 odontocetes varied from approximately 10 % of the total length of the complement in *Globicephala macrorhyncha* to approximately 15 % in *Tursiops gilli*. In the 3 balaenopterids, the amount of C-heterochromatin constituted approximately 25 % in the minke and sei whales and 30 % in the fin whale. Fig. 2 shows a C-banded karyotype of a female fin whale. The largest heterochromatic block of the fin whale karyotype was in the long arm of X. The relative length of the facultative heterochromatin of the X in this species was slightly less than 5 %, virtually the same as in the two other balaenopterids. The slightly lower value of relative length of the X chromosomes of the balaenopterids as compared with the odontocetes may be due to the greater amount of autosomal heterochromatin in the former.

The distribution of C-heterochromatin showed the same general pattern in the 7 species. The C-bands were mainly interstitial or terminal and to a lesser extent centromeric. In all species, size heteromorphism of heterochromatic blocks was observed. This heteromorphism was constant in all cells of the same specimen. A characteristic C-band pattern, including cases of size heteromorphism between homologues, is shown in Fig. 2.

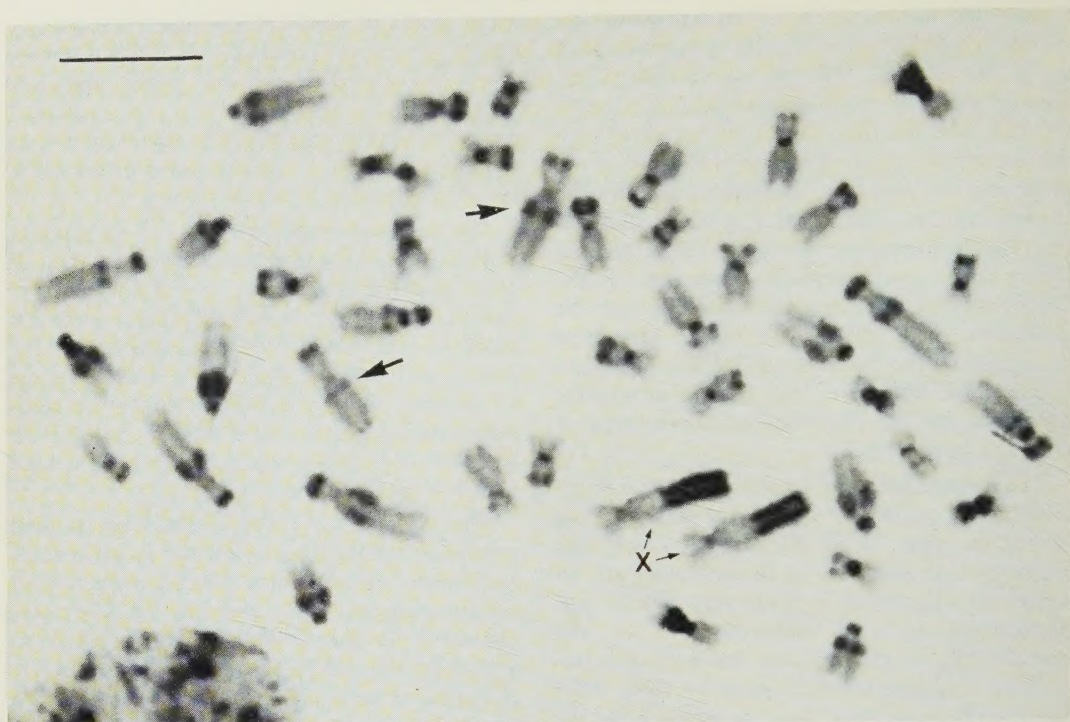


Fig. 2. C-banded metaphase chromosomes of a female fin whale, *Balaenoptera physalus*. One large autosomal pair showing C-band heteromorphism and the X chromosomes are indicated by arrows. Scale: 10 μ .

In all species, the distribution of terminal C-bands followed a general pattern. Thus, in the sm and st centromeric groups terminal C-bands were strictly limited to the short arms. In the t group terminal heterochromatin was present in one species only, the sei whale, which had terminal heterochromatin in two t pairs. In the m group, terminal C-heterochromatin was pronounced in the balaenopterid karyotypes, occurring mainly in the long arm of the chromosomes.

The cumulative evidence of the morphological agreement between the odontocete and mysticete karyotypes and the concordant features of the C-band pattern was interpreted as unequivocally supporting the theory of a monophyletic evolution of the Odontoceti and Mysticeti.

The striking heteromorphism of the C-heterochromatin in the cetacean karyotypes indicates

that structural rearrangements may occur within the heterochromatic blocks; however, the significance of the heteromorphism can only be conjectured. The presence of interstitial heterochromatic blocks of the same size may facilitate recombination in the adjacent euchromatin, as the repetitive composition of the heterochromatin would predispose to an early pairing of the blocks. On the other hand, interstitial blocks of markedly different sizes may render recombination of the adjacent euchromatin more difficult. The distribution of telomeric C-heterochromatin may be relevant for chromosome positioning during the cell cycle.

The large amounts of C-heterochromatin and the high degree of C-band heteromorphism in the cetacean karyotypes necessitate great caution in interspecific comparisons of chromosome measurements.

Conclusions

The exceptional karyotype uniformity and stability of the 2 orders, Pinnipedia and Cetacea, was discussed in an earlier paper (ÁRNASON 1972). In this connection it should be borne in mind that according to palaeontological findings, cetaceans with pronounced odontocete and mysticete characteristics occurred as early as late Eocene (SLIJPER 1936). The oldest palaeontological findings of pinnipeds are from middle Miocene; these findings show that even at this stage the otariids and phocids were well developed pinnipeds (KING 1964).

ÁRNASON (1972) associated the karyotypic uniformity of Pinnipedia and Cetacea with the following factors, characteristic of both orders:

- (1) Low prolificity
 - a) Late sexual maturity
 - b) Not more than one pup per year
- (2) Good vagility
- (3) Environment without delimited niches

The above factors may be taken as the principal causes of extreme karyotype stability, as the chances are remote that specimens having identically rearranged karyotypes will become the founders of new karyotypic subpopulations.

Conversely, the terrestrial mammalian orders Rodentia and Insectivora show great variation in karyotypic patterns, between and even within species. It is suggestive that in the case of Rodentia and Insectivora the relevant factors are in strong contrast with those listed above for Pinnipedia and Cetacea. Rodentia and Insectivora are characterized by:

- (1) High prolificity
 - a) Early sexual maturity
 - b) Large litters, many litters per year
- (2) Restricted vagility
- (3) Environment with delimited niches

All the above factors should contribute to a stronger tendency to inbreed in Rodentia and Insectivora as compared with Cetacea and Pinnipedia and thus give rise to karyotype variability and frequent establishment of subpopulations with new karyotypes.

The karyotypic uniformity within the Ceta-

cea and Pinnipedia shows that allopatric speciation prevails within these orders, whereas stasipatric speciation (WHITE 1968, 1973) is frequent in Rodentia and Insectivora.

The common occurrence of karyotype variants within the cetacean species and the rigid karyotype pattern in the pinnipeds were clearly correlated with the presence of large amounts of C-heterochromatin in the cetacean karyotypes, whereas C-heterochromatin is sparse in the pinniped karyotypes. Apparently, this trait has been preserved during the evolution of the Cetacea, while in Pinnipedia it was largely eliminated before their aquatic evolution, provided it was present at all in their terrestrial ancestors.

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